PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2006) **273**, 1111–1116
doi:10.1098/rspb.2005.3325
Published online 31 January 2006

Complex *Mhc*-based mate choice in a wild passerine

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The extreme polymorphism of the vertebrate major histocompatibility complex (*Mhc*) is famous for protecting hosts against constantly evolving pathogens. Mate choice is often evoked as a means of maintaining *Mhc* variability through avoidance of partners with similar *Mhc* alleles or preference for heterozygotes. Evidence for these two hypotheses mostly comes from studies on humans and laboratory mice. Here, we tested these hypotheses in a wild outbred population of house sparrows (*Passer domesticus*). Females were not more or less closely related to the males they paired with when considering neutral genetic variation. However, males failed to form breeding pairs when they had too few *Mhc* alleles and when they were too dissimilar from females at *Mhc* loci (i.e. had no common alleles). Furthermore, pairs did not form at random as *Mhc* diversity positively correlated in mating pairs. These results suggest that mate choice evolves in response to (i) benefits in terms of parasite resistance acquired from allelic diversity, and (ii) costs associated with the disruption of co-adapted genes.

Keywords: *Mhc*; mate choice; outbreeding avoidance; inbreeding avoidance; heterozygote advantage

1. INTRODUCTION

Mate choice is often evoked as a means of maintaining major histocompatibility complex (*Mhc*) variability through preference for heterozygotes (Doherty & Zinkernagel 1975; Landry *et al.* 2001; Arkush *et al.* 2002; Penn *et al.* 2002; McClelland *et al.* 2003), or avoidance of partners with similar *Mhc* alleles (Yamazaki *et al.* 1976, 1988; Potts *et al.* 1991; Penn & Potts 1998; Freeman-Gallant *et al.* 2003). Such preference may be better understood in the light of two commonly cited indirect benefits of mate choice, the search for good genes for the progeny (Hamilton & Zuk 1982) and inbreeding avoidance (Yamazaki *et al.* 1976, 1988; Potts *et al.* 1991; Penn & Potts 1998; Freeman-Gallant *et al.* 2003). Less attention has been devoted to the risk of outbreeding depression (Thornhill 1993), despite the fact that disruption of co-adapted genes may result in a severe fitness reduction (Hendry *et al.* 2000; Neff 2004).

The *Mhc* genes are particularly relevant for assessing whether mate choice is based on good genes, inbreeding or outbreeding avoidance. This multiloci gene complex codes for highly polymorphic molecules involved in defence against invading pathogens (Bodmer 1972; Carrington *et al.* 1999; Klein *et al.* 1993). As a result, individuals with either maximal numbers of *Mhc* alleles (heterozygote advantage) and/or with specific *Mhc* alleles (frequency-dependent selection) are privileged in a host population under pathogenic pressures (Doherty & Zinkernagel 1975; Hill 1998; Landry *et al.* 2001; Langefors *et al.* 2001; Arkush *et al.* 2002; Penn *et al.* 2002; McClelland *et al.* 2003). A female may therefore benefit from mating with a particular partner if she acquires 'resistance' genes for her progeny. *Mhc* genes are also known to be markers of the degree of relatedness between individuals, and a few studies have shown that *Mhc*-based mate choice may reflect the avoidance of incestuous mating rather than the search for 'good genes' (Yamazaki *et al.* 1976, 1988; Potts *et al.* 1991; Brown & Eklund 1994; Penn & Potts 1998).

Each of these hypotheses leads to testable predictions: (i) if there is a selective advantage of carrying many different alleles, then we expect the most diverse individuals to be preferred over the less diverse ones during pair formation; (ii) if inbreeding avoidance is the main force driving mate preference, we expect pairs to be assembled of more dissimilar individuals than found at random; and finally, (iii) if mate choice aims at preserving the linkage of co-adapted genes, we expect mates to share more alleles than found at random.

We tested these hypotheses in a wild outbred population of house sparrows (*Passer domesticus*). All individuals were screened both at neutral microsatellite loci and at the peptide-binding region of the most polymorphic gene family of *Mhc* class I (Bonneaud *et al.* 2004*a*). Previous work on this and another population showed that (i) *Mhc* diversity positively correlates with reproductive success (Bonneaud *et al.* 2004*b*), and (ii) specific *Mhc* alleles are associated with stronger immune responses to T-dependent antigens (Bonneaud *et al.* 2005),

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Received 18 July 2005
Accepted 4 September 2005

and confer increased resistance to malaria parasites (Bonneaud *et al.* in press). These results suggest that *Mhc* genes may be under intense selection in the house sparrow.

2. MATERIAL AND METHODS

(a) *The population*

This study was conducted during spring 2003 at the 'Centre d'Etude Biologique de Chizé' (France), on a nest-box house sparrow population established in 1992 (Chastel *et al.* 2003). Breeding males and females were captured at the nest-box when chicks were 8 days old. Breeding individuals that we failed to capture at nest, as well as non-mated individuals, were captured with mist nets permanently set up throughout the breeding season. Here we define mate choice as the selection of a social mate, and mating success as the outcome of pair formation. Non-mated males and females are therefore individuals who have failed to form a breeding pair. We measured body mass ($\pm$0.1 g), tarsus length ($\pm$0.01 mm) and male badge size ($\pm$0.01 mm, adult males only) for all captured adults and nestlings (when they were 10 days old). In addition, blood was sampled (*ca* 150 µl) and stored in PBS/EDTA 2 mM buffer at -20 °C.

(b) Mhc *screening*

We screened all individuals (adults and chicks) to assess allelic diversity at the most variable *Mhc* class I gene family using the PCR-based denaturing gradient gel electrophoresis (DGGE) method (Bonneaud *et al.* 2004*a*). This method allows us to examine single-nucleotide polymorphism at *Mhc* class I exon 3, corresponding to the highly variable peptide binding site of the protein (α2 domain). The PCR primers used were GCA21M-fA23M. Each DGGE band is considered to correspond to one allele (Bonneaud *et al.* 2004*a*).

This genotyping method does not allow us to determine the level of heterozygosity present at each individual locus. Instead, it gives us an estimate of the overall number of alleles present in the most variable lineage of *Mhc* class I genes. Individuals who carry the largest numbers of *Mhc* alleles are considered to be the most diverse. Although there seems to be a maximal number of six *Mhc* loci (the most diverse individuals have 11 alleles), we cannot rule out the possibility that we may be amplifying a gene family encompassing more than six loci.

(c) *Microsatellite analyses*

Adults were genotyped using seven microsatellite markers: Pdo3, Pdo4, Pdo5, Pdo6 (Griffith *et al.* 1999), Mjg1 (Shou-Hsien *et al.* 1997), Fhu2 (Primmer *et al.* 1996) and Ase18 (Richardson *et al.* 2000). Amplifications were run in a final volume of 10 µl, including 15–50 ng of DNA, 50–200 nM of each primer, 300 µM of dNTPs, 1 µl of 10$\times$ incubation buffer (50 mM KCl, 10 mM Tris–HCl, 1.5 mM $MgCl_2$, 0.1% TritonX-100, pH 9.0) and 0.25 U of *Taq* DNA polymerase (Qbiogene). The reaction was performed in a Gene Amp PCR System 9700 thermocycler (Applied Biosystems). Samples were then run in an ABI 310 automated sequencer (Applied Biosystems). Allele sizes were determined using GENESCAN software v. 2.1 with reference to the GENESCAN ROX 500 size standard.

(d) *Population structure and measures of relatedness*

Standard diversity indices, molecular indices and test of Hardy–Weinberg equilibrium were performed using Arlequin v. 2.0 software (Schneider *et al.* 2000). In addition, we used Guo & Thompson's method (1992) to detect significant departure from Hardy–Weinberg equilibrium (Fisher's exact test with the Markov chain method (chain length = 10 000 and dememorizations steps = 10 000)). Coefficients of relatedness (r) based on microsatellite genotype similarity were calculated between all male and female pairs using the KINSHIP program (Goodnight & Queller 1999). Pairwise relatedness values between all individuals of the population were also estimated to determine whether the population was inbred or outbred. Finally, mean individual heterozygosity was calculated across all loci.

(e) *Statistical analyses*

The probability of forming a breeding pair was modelled using a generalized linear model with number of *Mhc* alleles, body mass and tarsus length as continuous variables and sex as a factor (SAS institute 1999). *Mhc* similarity between females and males were calculated as *Mhc* band-sharing values; the proportion of band-sharing in a pair is twice the number of the bands shared by two individuals divided by the sum of the bands of each individual [$D = 2F_{ab}/(F_a + F_b)$] (Wetton *et al.* 1987). Band-sharing and relatedness values were analysed using a randomization test (Manly 1997). The observed median value of band-sharing and relatedness in breeding pairs was compared with the value obtained by 10 000 bootstrapped band-sharing and relatedness medians.

Spatial autocorrelation of number of *Mhc* alleles was assessed using Moran's I autocorrelation coefficients (R Package, v. 4.0; see http://www.bio.umontreal.ca/Casgrain/en/labo/R/).

3. RESULTS

We assessed mate choice in 45 female and 56 male adult house sparrows. Only 30 individuals of each sex successfully formed a breeding pair over the entire reproductive season (out of 37 breeding attempts, each involving different pairs of individuals).

(a) *Microsatellite genotyping*

The number of alleles per locus was 17 for Pdo3, 24 for Mjg1, 85 for Pdo4, 14 for Fhu2, 16 for Pdo5, 15 for Ase18 and 70 for Pdo6. The mean number of pairwise differences between all pairs of haplotypes was 6.321 ± 3.010 alleles and the average gene diversity was 0.903 ± 0.476. We found a significant deficiency in heterozygotes in three loci (Pdo4: $p < 0.0001$; Fhu2: $p < 0.0001$; Pdo6: $p = 0.045$). Females were not more or less related to their mates than found at random (randomization test: observed median relatedness value $= -0.014$, median of 10 000 bootstrapped band-sharing values $= -0.018$, $p > 0.05$). Negative pairwise values of relatedness indicate that the individuals were less genetically alike than found on average. In addition, relatedness values between mated pairs did not significantly differ from those obtained when pairing females and non-breeding males (randomization test: observed median band-sharing value $= -0.014$, median of 10 000 bootstrapped band-sharing values $= -0.009$, $p > 0.05$). Furthermore, it was found that mated males were not more

heterozygous across all loci than non-mated males (mated males = 0.114 ± 0.020; non-mated males = 0.120 ± 0.018; Student's $t_{78} = -0.20$, $p = 0.841$). Finally, calculation of overall pairwise population relatedness showed that this population was outbred (median = −0.017, mean = $6.627 \times 10^{-5} \pm 0.131$).

(b) Mhc-*based mate choice*

We found a total of 46 *Mhc* alleles present in 1–50% of the population, and individuals exhibited between 1 and 11 alleles (mean ± s.e., males = 3.48 ± 2.39, females = 3.40 ± 1.90, ANOVA: $F_{1,99} = 0.04$, $p = 0.852$). We tested whether the occurrence of each allele was independent of others in a restricted sample of the most frequent *Mhc* alleles (range 20–51%, $n = 6$). Analysis of contingency tables indicated that two pairs of *Mhc* alleles occurred together more frequently than expected at random (alleles *a161*–a165, $p = 0.0006$; alleles *a172*–a163, $p = 0.0093$).

Males that succeeded in forming a breeding pair displayed higher *Mhc* allele diversity than non-mated ones ($\chi^2 = 6.63$, $p = 0.010$; figure 1), but females did not ($\chi^2 = 0.77$, $p = 0.381$). This resulted in a statistically significant sex by allelic diversity interaction ($\chi^2 = 3.85$, $p = 0.0497$). However, the association between *Mhc* diversity and probability of forming a breeding pair was not due to the confounding effect of body condition since neither body mass nor tarsus length were retained in the model ($p = 0.524$ and $p = 0.625$, respectively).

Within the breeding population, pairs were not assembled of more dissimilar individuals than found at random (randomization test: observed median band-sharing value = 0.2, median of 10 000 bootstrapped band-sharing values = 0.182, $p = 0.306$; figure 2). Instead, females were paired to mates with whom they shared more *Mhc* alleles, as shown by the significant difference in the band-sharing values between females and breeding males versus non-breeding males (randomization test: observed median band-sharing value = 0.2, median of 10 000 bootstrapped band-sharing values = 0, $p = 0.006$; figure 3). Importantly, random pairs of males and females had an extremely low probability of carrying identical *Mhc* genotypes (0.3%), whereas they had a 54% chance of possessing completely different sets of alleles (i.e. band-sharing = 0).

The numbers of *Mhc* alleles found in males and females were significantly correlated (Spearman's $r = 0.371$, $p = 0.024$, $n = 37$). This non-random configuration of mating events affected the average number of *Mhc* alleles in chicks, so that highly diverse pairs produced highly diverse broods of chicks (slope ± s.e. = 0.523 + 0.129, $p = 0.0003$, $n = 34$). The mean allelic diversity did not differ between adults (mean ± s.e. = 3.45 ± 2.18) and offspring (mean ± s.e. = 3.39 ± 1.79, Student's $t_{174} = 0.21$, $p = 0.834$).

Mhc-based mate selection implies that *Mhc* genotypes can be predicted by phenotypic traits used as cues by the choosing partner. Nevertheless, we failed to show any significant correlation between *Mhc* genotypes and body mass and tarsus length in males and females, or the expression of a male secondary sexual trait (badge size; all $p > 0.1$). The house sparrow is a colonial nesting species with individuals aggregating at variable levels of density around nesting sites. We therefore tested whether a spatial aggregation of breeding individuals could generate the observed assortative mating. Coefficients of spatial autocorrelation did not, however, indicate any particular structuring of males, females or pairs in relation to their number of *Mhc* alleles (Moran's *I*, all $p > 0.1$).

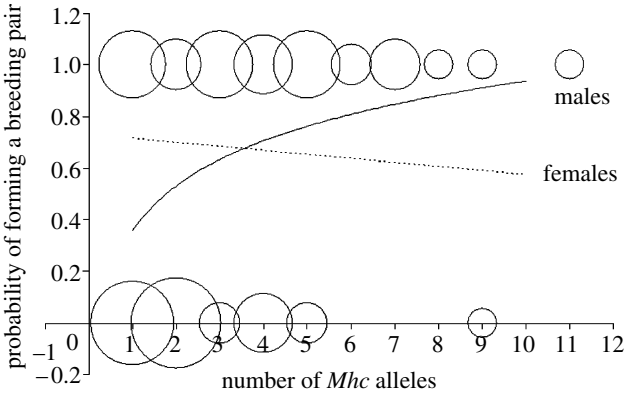

Figure 1. Probability of forming a breeding pair as a function of the number of *Mhc* alleles for both males (solid line) and females (dotted line). Probabilities of forming a breeding pair have been modelled using a logistic regression model. Observed values of male pairing success are represented by circles that vary in size according to the number of observations ($n = 1$, 2, 3, 4 or 5).

4. DISCUSSION

The mating patterns observed in this study strongly promote the idea of *Mhc*-related mate choice in birds. Mate choice in house sparrows proved to be based not only on the partners' allelic diversity at *Mhc* loci, but also on the number of shared *Mhc* alleles. As a result, males of low *Mhc* diversity that were too dissimilar from females at *Mhc* loci (i.e. no common alleles) were excluded from reproductive events. Moreover, *Mhc* diversity was positively correlated in both mating partners. Importantly, variation at neutral microsatellite markers did not influence reproductive decisions, suggesting that variation at *Mhc* loci *per se* may be driving mate choice in this population.

It is currently debated whether selection favours an intermediate or a maximum number of *Mhc* alleles (Penn *et al.* 2002; McClelland *et al.* 2003; Hedrick 2004; Wegner *et al.* 2004). An optimum number of *Mhc* alleles could be generated by a trade-off between selection for recognition of the largest antigenic peptide repertoire (Carrington *et al.* 1999; Arkush *et al.* 2002; Penn *et al.* 2002; McClelland *et al.* 2003), which favours more alleles, and selection to minimize the loss of T cell clones due to self-tolerance induction (Nowak *et al.* 1992), which favours fewer. By excluding males that are too dissimilar (i.e. band-sharing = 0), female house sparrows may prevent diversity from reaching detrimental levels in their offspring. Yet, by giving preference to mates with high allelic diversity, they may simultaneously endeavour to maximize the number of *Mhc* alleles in their progeny.

Rejection of the most dissimilar males may, however, have a larger significance than just avoiding costs of unfavourable diversity at the *Mhc*. Mating between highly divergent partners may disrupt local adaptations or co-adapted gene complexes (Hendry *et al.* 2000) and produce offspring of lower fitness. Furthermore, intermediate levels of genomic divergence have been shown to be

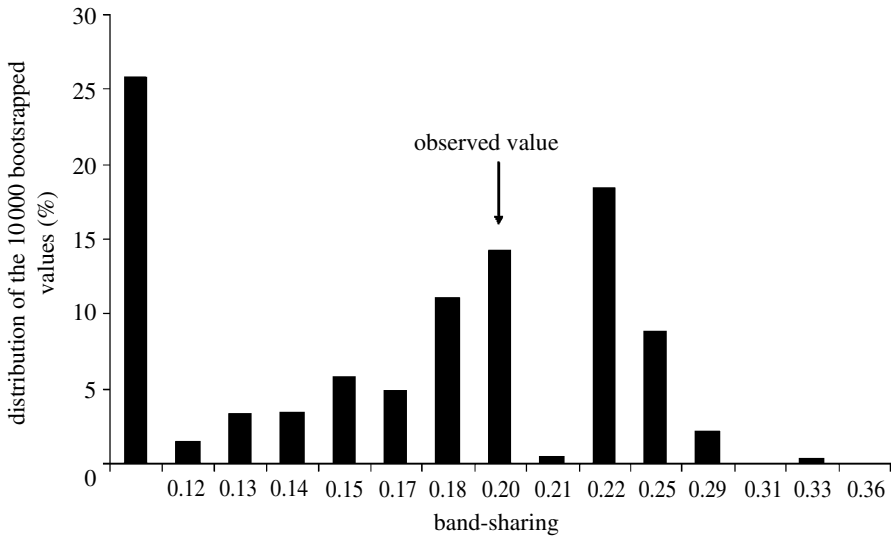

Figure 2. Distribution of the 10 000 bootstrapped *Mhc* band-sharing values for pairs assembled at random from the whole male population (i.e. mated and non-mated males). The observed value indicates the band-sharing value observed in true breeding pairs.

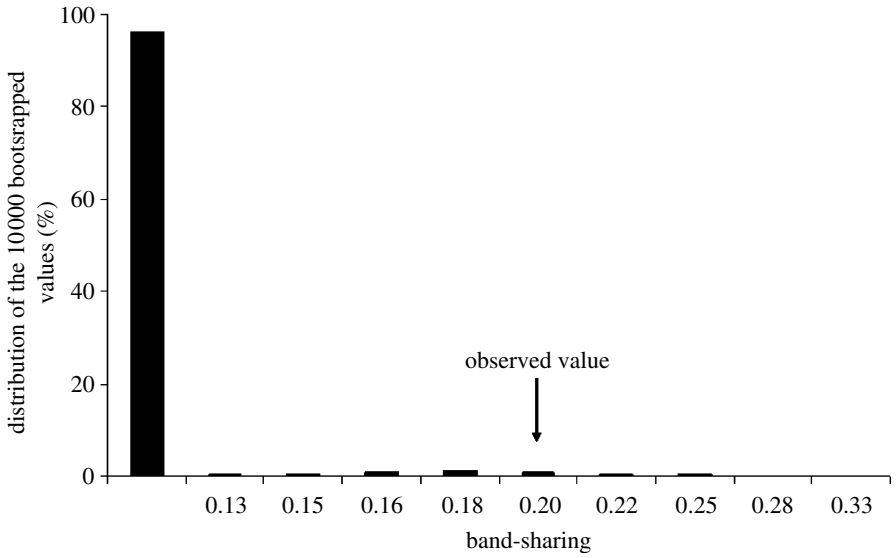

Figure 3. Distribution of the 10 000 bootstrapped *Mhc* band-sharing values for pairs composed solely of females and non-mated males. The observed value indicates the band-sharing value observed in true breeding pairs.

associated with higher reproductive success than more extreme levels of divergence (Neff 2004). The optimal level of genomic divergence may hence vary according to whether it is more advantageous to produce higher-quality surviving inbreds despite the increased mortality of offspring.

The *Mhc* alleles *a172* and *a163* were found to be significantly associated with each other. In a previous study involving this population of wild house sparrows, the allele *a172* was found to be associated with higher resistance to infections with the most common local *Plasmodium* strain, whereas none of the individuals that carried the *a163* allele endured simultaneous infections with two strains of malaria parasites (Bonneaud *et al.* 2005). We can easily speculate that rupture of this association of alleles would be selectively detrimental to the individual in terms of increased susceptibility to malaria infections. Co-adapted genes therefore exist in this population and probably undergo both parasite-mediated selection and reproductive selection.

Analyses of relatedness at neutral microsatellite loci showed that this population was outbred. In addition, mate choice was found to be based uniquely on *Mhc* genotypes, as females were not more or less closely related to the subset of males they paired with. Females only had 0.03% chances of pairing with a male carrying an identical combination of microsatellite alleles and 8% chances of pairing with a completely different male, so the absence of microsatellite-based reproductive preferences may be explained by a lack of inbreeding or outbreeding risks at neutral loci.

Our study reveals that females favoured males of comparable *Mhc* diversity and with higher numbers of matching alleles than found at random. It is fundamental to point out that females did not select males with identical *Mhc* genotypes, but rather excluded males with whom they shared no alleles. Hence our findings do not contradict previous work reporting avoidance of *Mhc*-similar mates (Yamazaki *et al.* 1976, 1988; Potts *et al.* 1991; Freeman-Gallant *et al.* 2003). Most experimental

assessments of mate choice have examined female preference between males of *Mhc* genotypes identical or different from their own. Yet, recent evidence suggests preference for mates with small or intermediate numbers of matching *Mhc* alleles rather than for mates with no or all matching alleles (Jacob *et al.* 2002). In this outbred population, the probability of pairing with a male of identical *Mhc* genotype was extremely low (0.3%), whereas the chance of randomly pairing with a male carrying a completely different set of *Mhc* alleles (i.e. band-sharing = 0) was 54%. A biased decision may allow females to control the number of *Mhc* alleles in their progeny and their overall level of genomic divergence, while still maximizing the number of *Mhc* alleles by favouring the most diverse males.

An underlying assumption to *Mhc*-based mate choice is that *Mhc* genotypes can be predicted by phenotypic traits used as cues by choosy partners. The direct implications of *Mhc* genes in fighting off diseases make obvious the links between *Mhc* genotypes and condition-dependent traits (Zelano & Edwards 2002). We failed to show any significant correlation between *Mhc* genotypes and body mass or tarsus length in males and females, or expression of a male secondary sexual trait (badge size). In the same way, coefficients of spatial autocorrelation did not indicate any particular structuring of males, females or pairs as a function of their number of *Mhc* alleles. Studies on *Mhc*-based mate choice in mammals and fish indicate that olfactory cues are generally used to discriminate among *Mhc* genotypes (Brown & Eklund 1994; Reusch *et al.* 2001). Because birds are commonly thought to be anosmatic, targets of sexual selection usually involve visual signals. Yet recent experimental work on different bird orders has revealed unsuspected abilities to discriminate between the odours of conspecifics and between those of aromatic plants (Petit *et al.* 2002; Hagelin *et al.* 2003; Bonadonna & Nevitt 2004). Association between the *Mhc*, odours, and mating preferences in birds is a promising question that awaits further exploration.

Mhc-based mate choice has now been reported in a variety of species throughout the vertebrate realm. Evidence in mammals (Yamazaki *et al.* 1976, 1988; Potts *et al.* 1991; Penn & Potts 1998; Jacob *et al.* 2002; but see Paterson & Pemberton 1997), fish (Landry *et al.* 2001; Reusch *et al.* 2001; Aeschlimann *et al.* 2003), reptiles (Olsson *et al.* 2003) and birds (Freeman-Gallant *et al.* 2003; but see Westerdahl 2004; Richardson *et al.* 2005) point towards a preference for the most heterozygous individuals or the use of *Mhc*-based cues to evaluate the degree of relatedness of a potential partner in order to avoid inbreeding or outbreeding. The optimal level of genomic diverge may however lie at more intermediate levels that maximize reproductive success (Thornhill 1993; Neff 2004). In this outbred population of house sparrows, female choice probably maintains a certain level of diversity in the offspring, while avoiding the costs resulting from the disruption of local adaptations.

We are grateful to Mark Kirkpatrick and Jean Clobert for their useful comments, to Céline Chastel for wonderful help in the field, and to John Ewen and Ryan Calsbeek for valuable advice. Financial support came from the Ministère de la Recherche (bourse doctorale to C.B. and ACI Jeunes Chercheurs to G.S.).

REFERENCES

Aeschlimann, P. B., Häberli, M. A., Reusch, T. B. H., Boehm, T. & Milinski, M. 2003 Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize *MHC* allele number during mate selection. *Behav. Ecol. Sociobiol.* **54**, 119–126.

Arkush, K. D., Giese, A. R., Mendonca, H. L., McBride, A. M., Marty, G. D. & Hedrick, P. W. 2002 Resistance to three pathogens in the endangered winter-run chinook salmon (*Oncorhynchus tshawyscha*): effects of inbreeding and major histocompatibility complex genotypes. *Can. J. Fish. Aquat. Sci.* **59**, 966–975. (doi:10.1139/f02-066)

Bodmer, W. F. 1972 Evolutionary significance of the HLA-system. *Nature* **237**, 139–145. (doi:10.1038/237139a0)

Bonadonna, F. & Nevitt, G. A. 2004 Partner-specific odor recognition in an Antarctic seabird. *Science* **306**, 835. (doi:10.1126/science.1103001)

Bonneaud, C., Mazuc, J., Chastel, O., Westerdahl, H. & Sorci, G. 2004*a* Terminal investment induced by immune challenge and *Mhc* associated fitness traits in the house sparrow. *Evolution* **58**, 2823–2830.

Bonneaud, C., Sorci, G., Morin, V., Westerdahl, H., Zoorob, R. & Wittzell, H. 2004*b* Diversity of *Mhc* class I and IIB genes in house sparrows (*Passer domesticus*). *Immunogenetics* **55**, 855–865. (doi:10.1007/s00251-004-0648-3)

Bonneaud, C., Richard, M., Faivre, B., Westerdahl, H. & Sorci, G. 2005 An *Mhc* class I allele associated to the expression of T-dependent immune response in the house sparrow. *Immunogenetics* **57**, 782–789.

Bonneaud, C., Pérez-Tris, J., Federici, P., Chastel, O. & Sorci, G. In press. *Mhc* alleles associated with local resistance to malaria in a passerine. *Evolution*.

Brown, J. L. & Eklund, A. 1994 Kin recognition and the major histocompatibility complex: an integrative review. *Am. Nat.* **143**, 435–461. (doi:10.1086/285612)

Carrington, M. *et al.* 1999 HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* **283**, 1748–1752. (doi:10.1126/science.283.5408.1748)

Chastel, O., Lacroix, A. & Kersten, M. 2003 Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus*. *J. Avian Biol.* **34**, 298–306. (doi:10.1034/j.1600-048X.2003.02528.x)

Doherty, P. C. & Zinkernagel, R. M. 1975 Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature* **256**, 50–52. (doi:10.1038/256050a0)

Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T. & Sollecito, S. V. 2003 Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Mol. Ecol.* **12**, 3077–3083. (doi:10.1046/j.1365-294X.2003.01968.x)

Goodnight, K. F. & Queller, D. C. 1999 Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol. Ecol.* **8**, 1231–1234. (doi:10.1046/j.1365-294x.1999.00664.x)

Griffith, S. C., Stewart, I. R. K., Dawson, D. A., Owens, I. P. F. & Burke, T. 1999 Extra-pair paternity in mainland and island populations of a socially monogamous bird, the house sparrow (*Passer domesticus*): is there an "island effect"? *Biol. J. Linn. Soc.* **68**, 303–316. (doi:10.1006/bijl.1999.0343)

Guo, S. & Thompson, E. 1992 Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.

Hagelin, J. C., Jones, I. L. & Rasmussen, L. E. L. 2003 A tangerine-scented social odour in a monogamous seabird. *Proc. R. Soc. B* **270**, 1323–1329. (doi:10.1098/rspb.2003.2379)

Hamilton, W. D. & Zuk, M. 1982 Heritable true fitness and bright birds: a role for parasites? *Science* **218**, 384–387.

Hedrick, P. W. 2004 Comment on "Parasite selection for immunogenetic optimality". *Science* **303**, 957a. (doi:10.1126/science.1092163)

Hendry, A. P., Burg, J. K., Bentzen, P., Volk, E. C. & Quinn, T. P. 2000 Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* **290**, 516–518. (doi:10.1126/science.290.5491.516)

Hill, A. V. S. 1998 The immunogenetics of human infectious diseases. *Annu. Rev. Immunol.* **16**, 593–617. (doi:10.1146/annurev.immunol.16.1.593)

Jacob, S., McClintock, M. K., Zelano, B. & Ober, C. 2002 Paternally inherited HLA alleles are associated with women's choice of male odor. *Nat. Genet.* **30**, 175–179. (doi:10.1038/ng830)

Klein, J., Satta, Y., O'hUigin, C. & Takahata, N. 1993 The molecular descent of the major histocompatibility complex. *Annu. Rev. Immunol.* **11**, 269–295. (doi:10.1146/annurev.iy.11.040193.001413)

Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. 2001 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proc. R. Soc. B* **268**, 1279–1285. (doi:10.1098/rspb.2001.1659)

Langefors, Å., Lohm, J., Grahn, M., Andersen, Ø. & von Schantz, T. 2001 Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. *Proc. R. Soc. B* **268**, 479–485. (doi:10.1098/rspb.2000.1378)

Manly, B. F. J. 1997 *Randomisation, bootstrap and Monte Carlo methods in biology*, 2nd edn. London: Chapman & Hall.

McClelland, E. E., Penn, D. J. & Potts, W. K. 2003 Major histocompatibility complex heterozygote superiority during coinfection. *Infect. Immun.* **71**, 2079–2086. (doi:10.1128/IAI.71.4.2079-2086.2003)

Neff, B. D. 2004 Stabilizing selection on genomic divergence in a wild fish population. *Proc. Natl Acad. Sci. USA* **101**, 2381–2385. (doi:10.1073/pnas.0307522100)

Nowak, M. A., Tarczy-Hornoch, K. & Austyn, J. M. 1992 The optimal number of major histocompatibility complex molecules in an individual. *Proc. Natl Acad. Sci. USA* **89**, 10 896–10 899.

Olsson, M., Madsen, T., Norby, J., Wapstra, E., Ujvari, B. & Wittzell, H. 2003 Major histocompatibility complex and mate choice in sand lizards. *Proc. R. Soc. B* **270**(Suppl. 2), S254–S256. (doi:10.1098/rsbl.2003.0079)

Paterson, S. & Pemberton, J. M. 1997 No evidence for major histocompatibility complex-dependent matings patterns in a free-living ruminant population. *Proc. R. Soc. B* **264**, 1813–1819. (doi:10.1098/rspb.1997.0250)

Penn, D. & Potts, W. 1998 *MHC*-disassortative mating preferences reversed by cross-fostering. *Proc. R. Soc. B* **265**, 1299–1306. (doi:10.1098/rspb.1998.0433)

Penn, D. J., Damjanovich, K. & Potts, W. K. 2002 *MHC* heterozygosity confers a selective advantage against multiple-strain infections. *Proc. Natl Acad. Sci. USA* **99**, 11 260–11 264. (doi:10.1073/pnas.162006499)

Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J. & Lambrechts, M. M. 2002 Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecol. Lett.* **5**, 585–589. (doi:10.1046/j.1461-0248.2002.00361.x)

Potts, W. K., Manning, C. J. & Wakeland, E. K. 1991 Mating patterns in seminatural populations of mice influenced by *MHC* genotypes. *Nature* **352**, 619–621. (doi:10.1038/352619a0)

Primmer, C. R., Møller, A. P. & Ellegren, H. 1996 New microsatellites from the pied flycatcher *Ficedula hypoleuca* and the swallow *Hirundo rustica* genomes. *Hereditas* **124**, 281–283. (doi:10.1111/j.1601-5223.1996.00281.x)

Reusch, T. B. H., Häberli, M. A., Aeschlimann, P. B. & Milinski, M. 2001 Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* **414**, 300–302. (doi:10.1038/35104547)

Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. & Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Mol. Ecol.* **9**, 2155–2234. (doi:10.1046/j.1365-294X.2000.105338.x)

Richardson, D. S., Komdeur, J., Burke, T. & von Schantz, T. 2005 MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proc. R. Soc. B* **272**, 759–767. (doi:10.1098/rspb.2004.3028)

SAS institute 1999 *SAS user's guide: statistics*, v. 6.12. Cary, NC: SAS institute.

Schneider, S., Roessli, D. & Excoffier, L. 2000 Arlequin: a software for population genetics data analysis. Version 2.0. Genetics and Biometry Lab, Department of Anthropology, University of Geneva.

Shou-Hsien, L., Yi-Jiun, H. & Brown, J. L. 1997 Isolation of tetranucleotide microsatellites from the Mexican jay *Aphelocoma ultramarina*. *Mol. Ecol.* **6**, 499–501. (doi:10.1046/j.1365-294X.1997.00215.x)

Thornhill, N. W. (ed.) 1993 *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*. Chicago, IL: University of Chicago Press.

Wegner, K., Kalbe, M., Kurtz, J., Reusch, T. B. H. & Milinski, M. 2004 Response to comment on "Parasite selection for immunogenetic optimality". *Science* **303**, 957b. (doi:10.1126/science.1093355)

Westerdahl, H. 2004 No evidence of an MHC-based female mating preference in great reed warblers. *Mol. Ecol.* **13**, 2465–2470. (doi:10.1111/j.1365-294X.2004.02238.x)

Wetton, J. H., Carter, R. E., Parkin, D. T. & Walters, D. 1987 Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* **327**, 147–149. (doi:10.1038/327147a0)

Yamazaki, K., Boyse, E. A., Mike, V., Thaler, H. T., Mathieson, B. J., Abbott, J., Boyse, J. & Zayas, Z. 1976 Control of mating preferences in mice by genes of the major histocompatibility complex. *J. Exp. Med.* **144**, 1324–1335. (doi:10.1084/jem.144.5.1324)

Yamazaki, K., Beauchamp, G. K., Kupniewski, J., Bard, J., Thomas, L. & Boyse, E. A. 1988 Familial imprinting determines H-2 selective mating preferences. *Science* **240**, 1331–1332.

Zelano, B. & Edwards, S. V. 2002 An *Mhc* component to kin recognition and mate choice in birds: predictions, progress, and prospects. *Am. Nat.* **160**, S225–S237. (doi:10.1086/342897)